Eur. J. Clin. Chem. Clin. Biochem.
Vol. 31, 1993, pp. 433–440

A New Look at the Limits of Detection (L_D), Quantification (L_Q) and Power of Definition (PD)

By *K. Gautschi*[1], *B. Keller*[2], *H. Keller*[1], *P. Pei*[1] and *D. J. Vonderschmitt*[1]

[1] *Institut für Klinische Chemie, Universitätsspital, Zürich, Schweiz*
[2] *Université Paris 7, UFR de Mathématiques 2, Paris*

(Received January 22/April 5, 1993)

Summary: The relationship between the concentration of the analyte and the imprecision of an analytical method can be displayed by the precision profile in which the coefficient of variation (relative standard deviation) is plotted against the concentration of the analyte. The function of the curve of the profile and its confidence limits can easily be assessed by a computer program developed by *W. A. Sadler & M. H. Smith* (Clin. Chem. *36* (1990), 1346–1350). For the assessment of limits of detection and of quantification the following procedure is proposed:

The lower (and upper) limit of the measuring interval is defined by the point at which an acceptable CV-line intersects the confidence limit. If, in the variance function one sets the concentration to zero, the normal distribution of the random errors of the blank will result. The mean of the next adjacent normal distribution, following the variance formula and overlapping the "zero-distribution" by a defined amount, represents the limit of detection. Within the described measuring interval, or within a fraction of it, one might construct overlapping normal distributions in an analogous manner. Their number represents the "power of definition" (PD) (instead of the "analytical sensitivity"), which also depends on the concentration of the determinand according to the variance function.

We tested these hypotheses by a comparison of two methods for the determination of cyclosporin A (ciclosporin, INN). Our results demonstrate that the data of the lower limits of the measuring interval and of the limit of detection agree well with data from the literature obtained in extensive interlaboratory surveys.

Introduction

The three most important characteristics of the efficiency of analytical methods, particularly in the field of instrumental chemical analysis, are[1]):

(i) limit of detection (L_D),

(ii) lower (and upper) limit of the quantification interval (LL_Q and UL_Q respectively), and

(iii) its "analytical sensitivity".

Instead of this ambiguous expression, we prefer the term "power of definition" (PD), characterizing the smallest difference of analyte concentration (or signal-difference) which can be discriminated with a defined statistical confidence.

Today, the definitions of limits for "... qualitative detection and quantitative determination ..." given by *Currie* (1) are widely accepted. The statistics of detection and determination deal specifically with the observed signal and its associated random fluctuations, in other words with the analytical imprecision, which *Currie* assumes to be constant (homoscedastic).

It is a common occurrence in many analytical assays that the distribution of random errors varies with the

[1]) List of abbreviations used:
L_D = Limit of detection
L_C = Critical limit
LL_Q = Lower limit of quantification interval
UL_Q = Upper limit of quantification interval
PD = Power of definition

concentration of the determinand, embedded in the test material (reviewed in l. c. (2)). This is virtually the rule with radioimmunoassays. Thanks to the systematic investigations of the dose response curves of various immunoassays by *Rodbard & Cooper* (3) the interdependence of imprecision and concentration is commonly appreciated today.

Bayer's concept (4) of the "lower limit of an interassay quantitative measurement" is based on this relation. A precision profile according to *Ekins* (5) is constructed and this limit is graphically assessed "... from the intercept of the interassay CV (y axis) equal to 10% or 15% with the precision profile curve ...".

The precision profile is an instructive graphical representation of the intra-assay (or interassay, or interlaboratory etc.) coefficient of variation (CV) versus the concentration of the analyte in an x/y diagram. If instead of the CV the analytical variance is plotted against the concentration, then the shape of the curve follows mathematically the "variance function". *Rodbard* (6), *Ekins* (7), and *Raab* (8) have developed indirect methods for their assessment, whereas *Baxter* (9), *Sadler* et al. (10) and *Raggatt* (11) have described direct methods for their calculation.

Theoretical Considerations

Recently *Sadler & Smith* (12) also published a method for computing and plotting the confidence intervals for precision profiles. As in an earlier paper (13) the construction of the profile is based on a three-parameter variance function:

$$\sigma^2(U) = (\beta_1 + \beta_2 U)^J$$

where $\sigma^2(U)$ denotes variance, U stands for concentration, and β_1, β_2, and J are the parameters. The confidence interval is assessed according to l. c. (14). Interval widths reflect both the quantity of data and the way the data are distributed over the concentration range.

For the assessment of limits of detection and of quantification the following inferences are suggested:

i) The lower (and upper) limit of the measuring interval might be more reliably defined, if the threshold corresponds to the point at which an acceptable CV-line (e. g. 10% or 15%) intersects the confidence limit, rather than the curve itself.

ii) If, in the 3-parameter variance function one sets the value for U to zero, the normal distribution of the random errors of the blank will result. The mean of the next adjacent normal distribution, following the variance formula and overlapping the "zero-distribution" by a defined amount, represents the limit of detection.

iii) Within the measuring interval, as described under (i), or within a fraction of it, one might construct overlapping normal distributions in an analogous manner. Their number represents the "power of definition" (PD) (instead of the "analytical sensitivity"), which also depends on the concentration of the determinand according to the variance function.

In order to test the validity of these statements, we shaped an artificial data base of 11 × 10 values[2]) as a model, following the function

$$s^2(U) = (0.1 + 0.05\ U)^{3.0}$$

The plot of variances versus concentration performed by the *Sadler-Smith*-programs is shown in figure 1. Obviously the eleven means with their corresponding curve are enveloped in an increasingly wider band of confidence limits. A precision profile results if, instead of the variance, the coefficient of standard deviation ($\sqrt{s^2}/U \times 100$) is scaled on the ordinate (fig. 2). The curve declines continuously from the point of the lowest concentration (0.5), reaching a minimum at the value of 4.0, and rises slowly afterwards. The

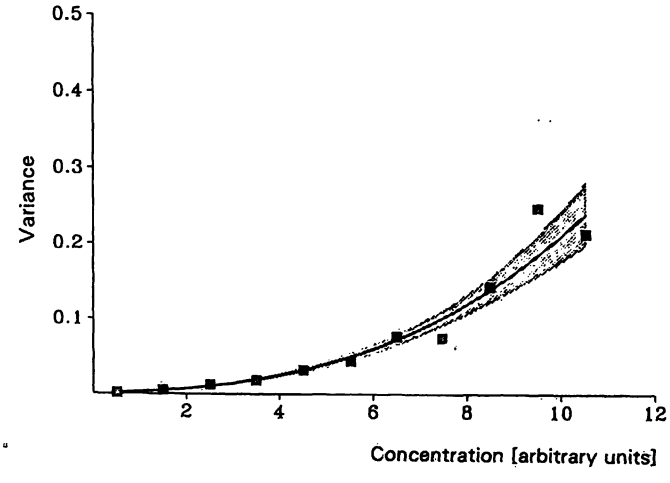

Fig. 1. Plot of variance (y-axis), as a function ($F \doteq (0.1 + 0.05\ U)^{3.0}$) of concentration (x-axis, arbitrary units) of a model population, and 95% confidence interval, adjusted for 100-fold measurements.

[2]) The model consists of 11 imaginary samples with means μ increasing from 0.5 to 10 in steps of 1. For each sample a hundred "measurements" are performed. They are the values of simulated independent random variables with distribution $N\{\mu, \sigma^2\}$, where $\sigma^2 = (0.1 + 0.05\ \mu)^3$. The variables were generated by *Box-Muller*'s transformations (p. 453 in l. c. (15)).

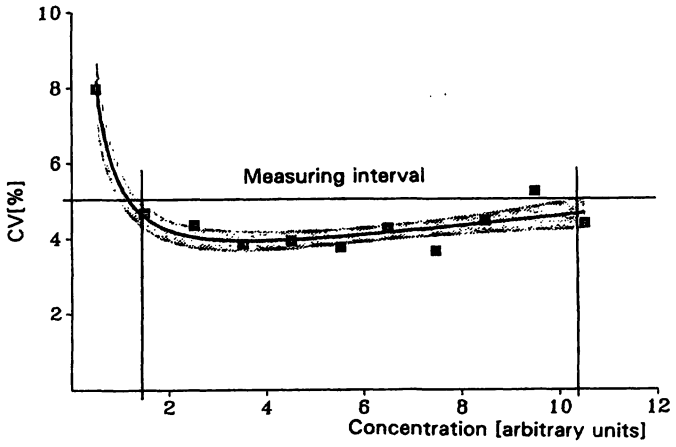

Fig. 2. As figure 1, the variance is substituted by CV on the y-axis. The measuring interval is given by the intersections of a 5.5% CV line with the lower and upper edge of the confidence interval of the CV curve.

confidence interval increases slowly. With an accepted CV of 5.0%, the measuring interval spreads from 1.4 to 10.4 arbitrary units.

The following procedure might be used to assess the limit of detection by means of the variance function according to statement (ii):

Given the mean μ and variance σ^2 we write $N\{\mu,\sigma^2\}$ for the corresponding normal distribution. Let L_D be the concentration for which $N\{L_D,\sigma^2(L_D)\}$ and $N\{0,\sigma^2(0)\}$ overlap by a given amount, e.g. 5% (fig. 3).

Let F be the given overlap, e.g. 5%. In order to determine L_D in practice, we solve the equation:

$$F = \Phi\{L_D,\sigma^2(L_D),L_C\} + (1 - \Phi\{0,\sigma^2(0),L_C\})$$

by an iterative numerical procedure (secant method (15)).

Here, for any μ, σ^2 and L_C, we express by $\Phi\{\mu,\sigma^2,L_C\}$ the area between $-\infty$ and L_C under the normal distribution $N\{\mu,\sigma^2\}$; and we denote by L_C the critical limit, i.e. the ordinate of the intersection of the normal distribution $N\{L_D,\sigma^2(L_D)\}$ and $N\{0,\sigma^2(0)\}$. We calculate L_C by the *Newton-Raphson* procedure (15).

If one accepts an overlap of 5%, the following values result from the model described:

$$\sigma(0) = 0.0316,\ \sigma(L_D) = 0.0348,$$
$$L_C = 0.0628,\ L_D = 0.1302.$$

The analytical power of definition (PD) within the measuring interval of the model can be assessed analogously: beginning with the concentration of 2.0 as the mean, the right half of a normal distribution is constructed, the next distribution which yields 5% overlapping is connected and so on, up to the concentration of 9.0 (fig. 4). Obviously 10 normal distributions can be inserted, when the overlapping is resticted to 5%. If an overlap of 10% would be tolerated, then 20 distributions would be possible.

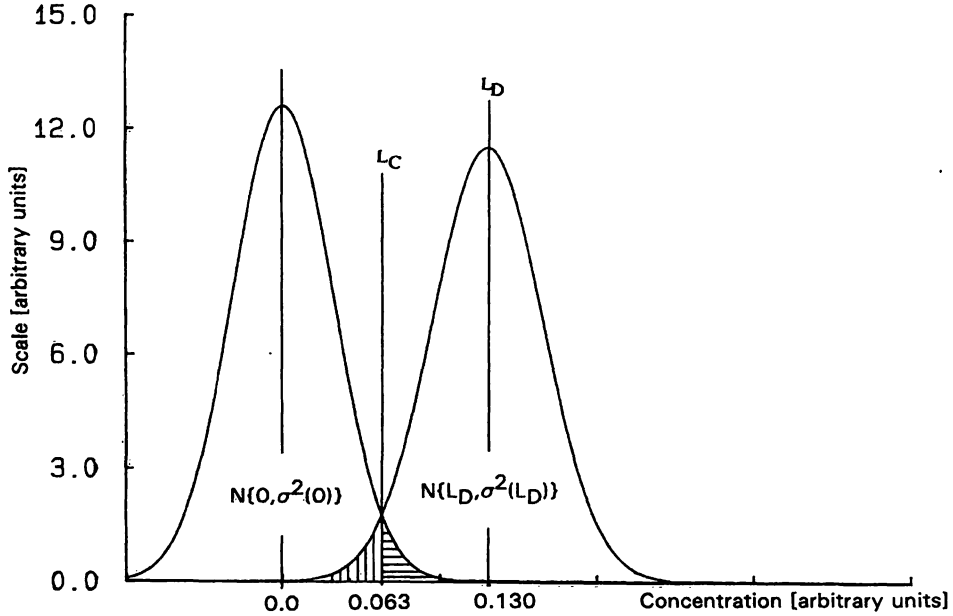

Fig. 3. Assessment of the limit of detection: first a normal distribution is constructed, whose standard deviation corresponds to $\sqrt{0.1^3} = 0.0316$ (analogous to the variance formula with U = 0); the next adjacent normal distribution following the variance formula and overlapping the zero-distribution by 5% is calculated by iterative numerical procedures; L_C: Critical Limit, L_D: Limit of Detection.
vertically hatched: $\Phi\{L_D,\sigma^2(L_D),L_C\}$
horizontally hatched: $1 - \Phi\{L_D,\sigma^2(L_D),L_C\}$

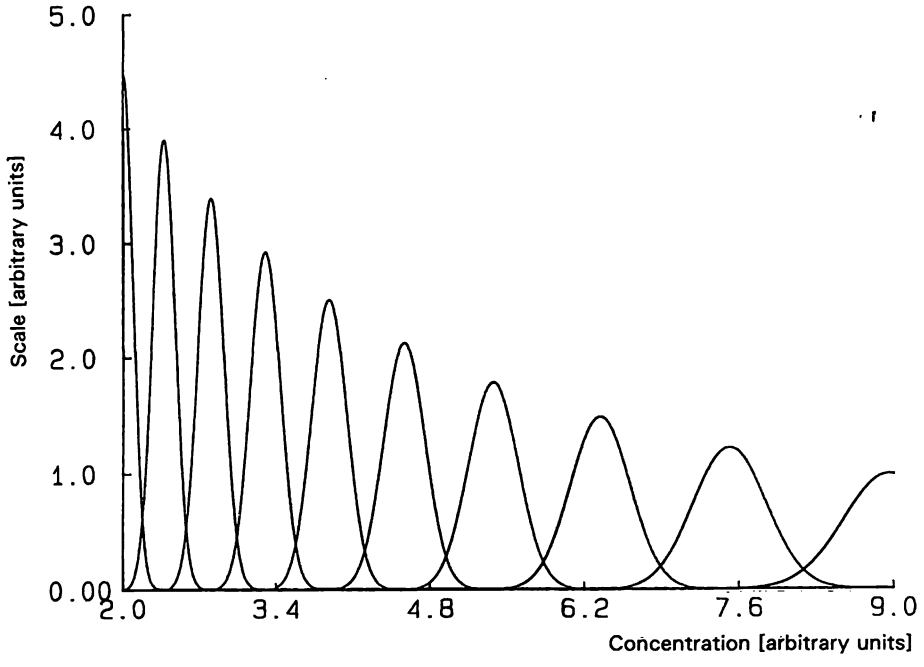

Fig. 4. Assessment of the power of definition: analogous to the procedure shown in figure 3: first the right half of a normal distribution with the mean value of 2.0 is constructed, whose standard deviation corresponds to $\sqrt{(0.1 + 0.05 \times 2)^3} = 0.089$; the next adjacent distributions with overlaps of 5% between neighboring distributions are added up to the concentration of 9.0 arbitrary units.

Practical Examples

A) Radioimmunoassay for the determination of ciclosporin in haemolysate (16)

In an evaluation of methods for the determination of the immunsuppressive, ciclosporin, we determined in analogous manner the lower limit of the measuring interval (LL_Q), limit of detection (L_D) and the power of definition (PD) of the immunoassay Cyclotrac SP (Incstar Corp.) RIA.

This assay is routinely used in our laboratory for ciclosporin determination in haemolysates from liver- or kidney-transplanted patients. Samples from patients, not treated with cyclosporin (blanks) were not investigated. Usually the assays are performed as triplicates. Out of the laboratory protocols of June–August 92, the results of 112 triplicates are selected. The used triplicate values are therefore within-assay-values, and the sum of all triplicates represents between-assay-estimates. This distinction corresponds to common usage in laboratory practice.

The 336 results were chosen randomly without elimination of outliers. The database was processed by the *Sadler-Smith*-program. The lowest measured value was 23 µg/l, the highest was 504 µg/l.

The variance function, according to *Sadler-Smith* was:

$$s^2(U) = (6.733 + 0.08957\ U)^{1.601}$$

Hence it follows:

i) Lower limit of measuring interval (fig. 5): if an imprecision of CV = 15% is tolerated, LL_Q is given by 57 µg/l; for a CV = 10% LL_Q is 105 µg/l; (the upper limit of the measuring interval (UL_Q) cannot be assessed by the diagram).

ii) Limit of detection (fig. 6): L_D equals 19.9 µg/l with a statistical confidence of $p \geq 0.95$; L_C is given by 9.3 µg/l.

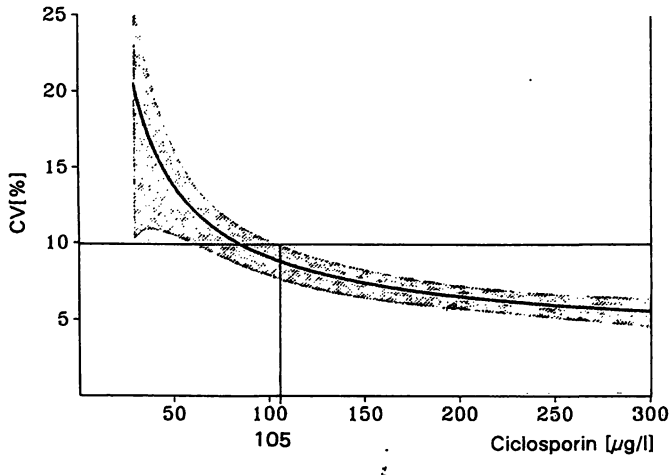

Fig. 5. Constructed as in figure 2 with the the variance function of the cyclosporin/RIA-assay: $s^2(U) = 6.73320 + 0.08957\ U)^{1.601}$.

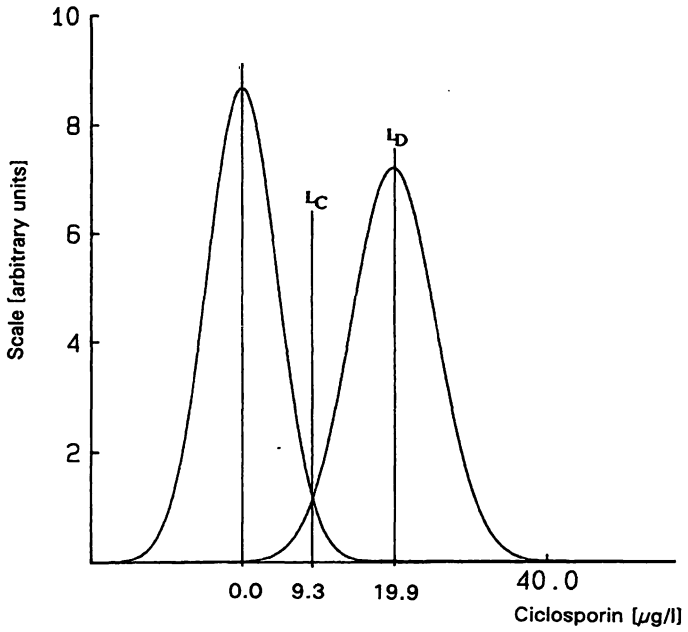

Fig. 6. Constructed as in figure 3 with the the variance function of the cyclosporin/RIA-assay: $s^2(U) = (6.73320 + 0.08957\ U)^{1.601}$.

iii) Power of definition (PD): within the concentration interval from 100 to 400 µg/l, the preferred therapeutic interval, it is possible to construct 6 normal distributions, following the variance function and with an overlap of 5% (fig. 7); the standard deviation increases from 9.06 µg/l for c = 100 µg/l (CV = 9.06%) to 19.9 µg/l for c = 400 µg/l (CV = 5.04%).

B) Fluorescence-polarization-immunoassay for the determination of ciclosporin in haemolysate (17)

We compared the analytical efficiency of the RIA with the widespread TDx-Cyclosporin monoclonal Abbott Laboratories. As with the RIA, we used the results of another 112 triplicates, collected in a random fashion from the printouts of the analyser, and processed without elimination of outliers. All haemolysates originated from patients after kidney or liver transplantation. Samples from patients not treated with ciclosporin (blanks) were not investigated. The lowest measured ciclosporin concentration was 46.3 µg/l, the highest was 601.8 µg/l.

The variance function, according to *Sadler-Smith* was:

$$s^2(U) = (1.25932 + 0.00105\ U)^{9.185}$$

hence it follows:

i) Lower limit of measuring interval, LL_Q (fig. 8): if an imprecision of CV = 10% is tolerated, LL_Q is given by 42 µg/l; for a CV = 5% LL_Q is 98 µg/l; (the upper limit of the measuring interval cannot be assessed by the diagram).

ii) Limit of detection (fig. 9): L_D equals 11.6 µg/l with a statistical confidence of $p \geq 0.95$; L_C is given by 5.7 µg/l.

iii) Power of definition (PD): within the concentration interval from 100 to 400 µg/l, it is possible

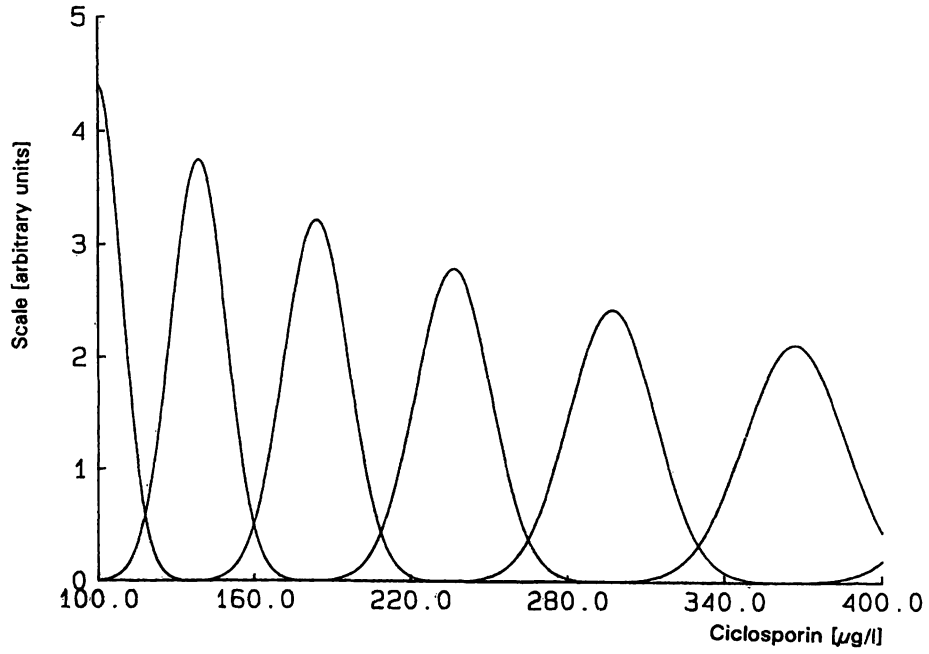

Fig. 7. Constructed as in figure 4 with the the variance function of the cyclosporin/RIA-assay: $s^2(U) = (6.73320 + 0.08957\ U)^{1.601}$.

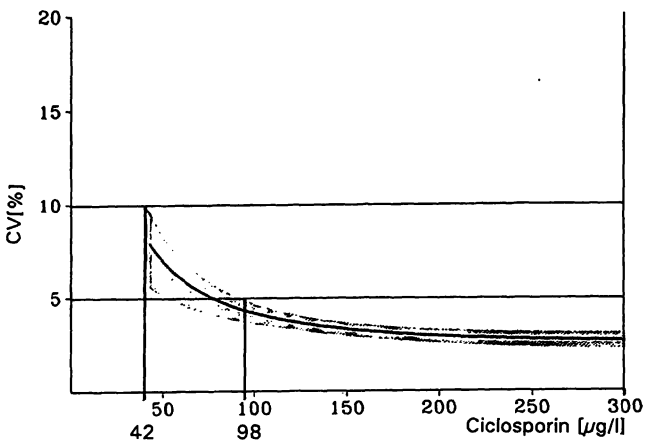

Fig. 8. Constructed as in figure 2 with the the variance function of the cyclosporin/TDx-assay:
$s^2(U) = (1.25932 + 0.00105\ U)^{9.185}$.

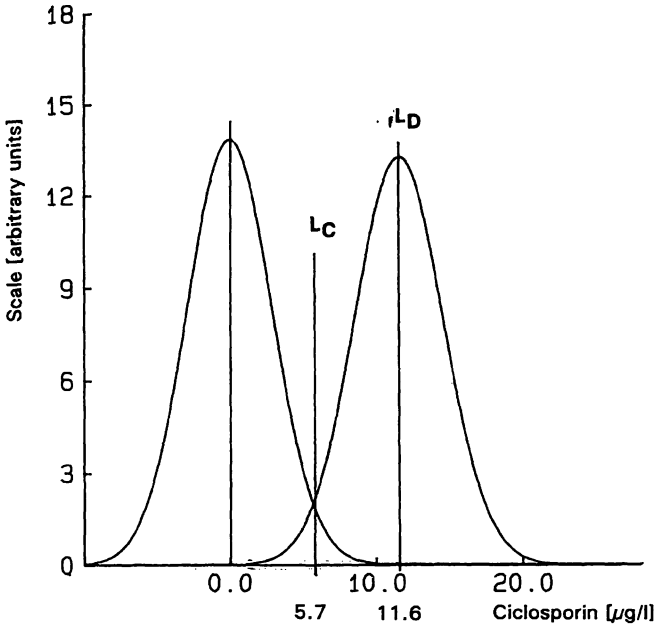

Fig. 9. Constructed as in figure 3 with the the variance function of the cyclosporin/TDx-assay:
$s^2(U) = (1.25932 + 0.00105\ U)^{9.185}$.

to construct 12 normal distributions, following the variance function and with an overlap of 5% (fig. 10); the standard deviation increases from 4.16 µg/l for c = 100 µg/l (CV = 4.16%) to 10.79 µg/l for c = 400 µg/l (CV = 2.70%).

Discussion

In order to assess the performance of *immunoassays* one can use the variance function according to *Ekins* (7) and *Sadler* et al. (18). Nevertheless, the dependence of the analytical variance on the concentration of the analyte is not restricted to immunoassays: precision profiles from the *Sadler-Smith*-program can also be constructed from data from the methodological comparisons of sodium determinations by flame-photometry and ion selective electrodes; or of measurements of creatinine by enzymatic and *Jaffé*-methods (2), or of determinations of cholesterol by conventional and dry-chemistry methods (19). In recent, unpublished studies we successfully evaluated methods for the determination of cadmium by atomic absorption spectrometry, or lithium with ion selective electrodes in the same way. The data, originating from patient-material as well as from artifically spiked solutions

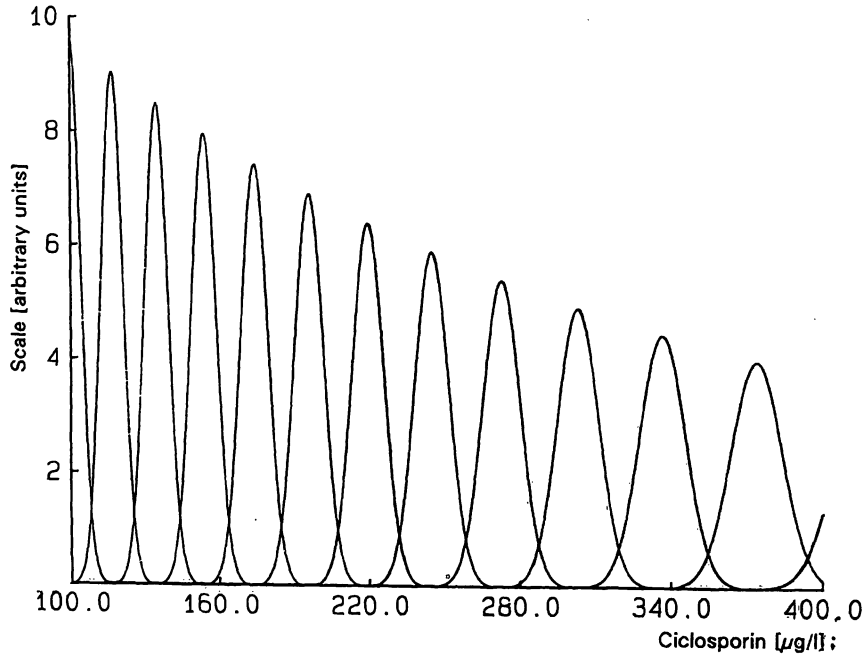

Fig. 10. Constructed as in figure 4 with the the variance function of the cyclosporin/TDx-assay:
$s^2(U) = (1.25932 + 0.00105\ U)^{9.185}$.

can all be processed by the *Sadler-Smith*-program without any problems, provided an adequate number of different measuring points is given.

Only a few authors (e. g. l. c. (4, 7, 19)) have followed the suggestion that the *limit(s) of the measuring interval* should be assessed by means of the precision profile or the variance function. Nevertheless, practical experience has shown that this provides the laboratory with an objective criterion for the evaluation and a powerful tool for the judgement of the performance of the methods used. The idea of defining the measuring interval as the intersection of a CV line with the upper edge of the CV curve confidence interval is decidedly a conservative approach. In defining the limits in this manner the efficiency of the method under investigation will certainly not to be overestimated.

The importance of a reliable assessment of the *limit of detection (L_D)* for diagnostic purposes is very high in laboratory medicine, e. g. for the verification of intoxications, screening of drugs, control of tumour marker concentrations, serological detection of antigens or antibodies etc. In order to determine L_D the majority of clinical laboratories perform multiplicate analyses of an analyte-free standard, and define L_D as the concentration that equals the value of three standard deviations above zero (20). This definition corresponds to the error distribution of the blank, it confines the tolerated α-error, and "... probably gives an overly optimistic estimate ... (21)" of L_D. Furthermore, a blank, the matrix of which corresponds almost completely to the actual samples, is often difficult or even impossible to obtain for biological material. The extrapolation from low values of real samples to the zero-value by means of the variance function seems to produce more realistic data.

For this very reason the following definition has been proposed (18):

$$L_D = Z \times SD/\sqrt{2},$$

where Z denotes the one-tailed standard normal deviate. It is important to emphasize that this definition fixes the magnitude of the β-error. The approximation of L_D by the described iterative procedures, however, makes it possible to fix the magnitude of the total error, e. g.

$$\alpha\text{-error} + \beta\text{-error} \leq 5\%$$

The fact that the calculation is more complicated is of no importance given the high performance of modern PCs.

Also, the following should be taken into consideration: in the range of low analyte concentrations all instruments of analytical methods necessarily produce negative signals occasionally. Modern analyser systems ("black boxes"), on the other hand, never produce negative results; many systems do not even produce zero-results. Instead, they indicate by a symbol that the result is below a certain lower measuring-limit, the value of which is rarely known to the user. In order to define the L_D for this kind of analytical systems, the assessment by means of the variance function seems to be the only possible way.

For the assessment of *power of definition (PD)* the bidirectional calculation is also desirable, in order to control α-errors and β-errors simultaneously. Together with the analytical variance (or STD or CV), the PD is always changing with changes of the concentration. The assessment of the PD can always be restricted to the measuring interval, or to an interval of diagnostic interest. Knowing the specific variance function and being able to perform the described iterative procedures, the laboratory can construct a special method-dependent scale, analogous to figures 4, 7, or 10 for this interval. In practice an output device might calculate the minimal distinguishable differences, so that the laboratory exclusively prints out and/or documents values that differ by a defined level of confidence.

Recently *Fraser* et al. (22), again pointed out that in order to recognize differences of concentrations at the 95% level of confidence a factor of 1.96 (or 2.58 at the 99% confidence level) is mandatory. This means that at a 10% CV, e. g. a difference of concentration of 20% (or 26%) is necessary for a firm distinction.

Encompassing their "UK Cyclosporin Quality Assessment Scheme" (23) *Holt* et al. (24) have evaluated the results of 176 participating laboratories; in summary, they stated "... the best precision was shown by the most automated methods, that is the FPIA TDx-SP...". Their judgement, based on an interlaboratory survey, agrees very well with the results of our interassay study where the imprecision of the automated assay is half of that for manual procedure.

Although ciclosporin has recently been prescribed in low doses for the treatment of autoimmuno diseases, one cannot find exact information about the limit of detection in the literature. A recent study of *Holt* et al. (25) has shown how important this knowledge might be: laboratories (up to 38% of the participants) reported partly high ciclosporin concentrations in a blank specimen (without ciclosporin), given in a qual-

ity assessment study. These false-positive results reflect the ignorance of many laboratories about the efficiency and performance of the methods they use.

Acknowledgement

We thank *Silvia Brogli* and *Yvonne Neumaier* for excellent technical support of our study.

References

1. Currie, L. A. (1968) Limits for Qualitative Detection and Quantitative Determination. Anal. Chem. *40*, 586–593.
2. Keller, H. & Passing, H. (1989) Performance Profiles: New Tools for Characterization and Comparison of Clinical Chemical Results. J. Clin. Chem. Clin. Biochem. *27*, 613–629.
3. Rodbard, D. & Cooper, J. A. (1970) A Model for Prediction of Confidence Limits in Radioimmunoassays and Competitive Binding Assays. In: *Proceedings of the Symposium*, Internat. Atomic Energy, Vienna, pp. 659–674.
4. Bayer, M. F. (1987) Performance Criteria for Appropriate Characterization of "(Highly) Sensitive" Thyrotropin Assays. Clin. Chem. *33*, 630–631.
5. Ekins, R. P. (1976) General Principles of Hormone Assay. In: *Hormone Assays and Their Clinical Application* (Lorraine, I. A. & Bell, E. T., eds.) pp. 2–72, Churchill Livingstone, Edinburgh.
6. Rodbard, D. (1974) Statistical Quality Control and Routine Data Processing for Radioimmunoassays and Immunoradiometric Assays. Clin. Chem. *20*, 1255–1270.
7. Ekins, R. P. (1983) The Precision Profile: its Use in Assay Design, Assessment and Quality Control. In: *Immunoassays for Clinical Chemistry*, 2nd ed. (Hunter, W. M. & Corrie, J. E. T., eds.) pp. 76–105, Churchill Livingstone, Edinburgh.
8. Raab, G. M. (1983) Validity Tests in Statistical Analysis of Immunoassay Data. In: *Immunoassays for Clinical Chemistry*, 2nd ed. (Hunter, W. M. & Corrie, J. E. T., eds.) pp. 614–623, Churchill Livingstone, Edinburgh.
9. Baxter, R. C. (1980) Simplified Approach to Confidence Limits in Radioimmunoassay. Clin. Chem. *26*, 763–765.
10. Sadler, W. A., Smith, M. H. & Legge, H. M. (1988) A Method for Direct Estimation of Imprecision Profiles, with Reference to Immunoassay Data. Clin. Chem. *34*, 1058–1061.
11. Raggatt, P. R. (1989) Duplicate or Singletons? – An Analysis of the Need for Replication in Immunosassay and a Computer Program to Calculate the Distribution of Outliers, Error Rate and the Precision Profile from Assay Duplicates. Ann. Clin. Biochem. *26*, 26–37.
12. Sadler, W. A. & Smith, M. H. (1990) Use and Abuse of Imprecision Profiles: Some Pitfalls Illustrated by Computing and Plotting Confidence Intervals. Clin. Chem. *36*, 1346–1350.
13. Sadler, W. A. & Smith, M. H. (1986) A Reliable Method of Estimating the Variance Function in Immunoassay. Comput. Statist. Data Analysis *3*, 227–239.
14. Bard, Y. (1974) *Non-Linear Parameter Estimation.* Academic Press, New York.
15. Dahlquist, G. & Björk, Å. (1974) *Numerical Methods.* Prentice-Hall, New Jersey; Newton-Raphson's method: p. 222, Secant Method: p. 227.
16. McBride, J. H., Rodgerson, D. O., Park, S. S. & Reyes, A. F. (1989) Measurement of Cyclosporin in Plasma from Patients with Various Transplants: HPLC and Radioimmunoassay with a Specific Monoclonal Antibody Compared. Clin. Chem. *35*, 1726–1730.
17. Yatscoff, R. W., Copeland, K. R. & Faraci, C. J. (1990) Abbott TDx Monoclonal Antibody Assay Evaluated for Measuring Cyclosporine in Whole Blood. Clin. Chem. *36*, 1969–1973.
18. Sadler, W. A., Murray, L. M. & Turner, J. G. (1992) Minimum Distinguishable Difference in Concentration: a Clinically Oriented Translation of Assay Precision Summaries. Clin. Chem. *38*, 1773–1778.
19. Keller, H. (1990) Vom Umgang mit Labordaten. Schweiz. Med. Wochenschr. *121*, 1861–1869.
20. Kaiser, H. (1965) Zum Problem der Nachweisgrenze. Z. Analyt. Chem. *209*, 1–18.
21. McConway, M. G., Chapman, R. S., Beastall, G. H., Tillman, J., Bonar. J. A., Hutchison, A., Allison, T., Tinlayson, J., Weston, R., Beckett, G. J., Carter, G. D., Carlyle, E., Herbertson, R., Blundell, G., Edwards, W., Glen, A. C. A. & Reid, A. (1989) How Sensitive Are Immunometric Assays for Thyrotropin? Clin. Chem. *35*, 289–291.
22. Fraser, C. G., Hylthoft Petersen, P. & Lytken Larsen, M. (1990) Setting Analytical Goals for Random Analytical Error in Specific Clinical Monitoring Situations. Clin. Chem. *36*, 1625–1628.
23. Johnston, A., Marsden, J. T. & Holt, D. W. (1986) The United Kingdom Cyclosporin Quality Assessment Scheme. Therap. Drug Monit. *8*, 200–204.
24. Holt, D. W., Marsden, J. T. & Johnston, A. (1990) Quality Assessment of Cyclosporine Measurement: Comparison of Current Methods. Transplant. Proceedings *22*, 1234–1239.
25. Johnston, A. & Holt, D. W. (1993) External Quality Assessment Scheme for Cyclosporin in Body Fluids. Scand. J. Clin. Invest. in press.

Prof. Dr. D. J. Vonderschmitt
Institut für Klinische Chemie
Universitätsspital
Rämistraße 100
CH-8091 Zürich